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Discovery of Peptidic Anti-cobratoxins by Next Generation Phage Display

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Figure 1: *Naja kaouthia* by S. Ganguly 2012

The future of antivenoms – synthetic antitoxins

Antivenoms are still being produced by animal immunization protocols and are therefore associated with high immunogenicity for human recipients [1]. Here we report the first step towards discovery of synthetic antitoxins that could be used for development of a fully synthetic antivenom against neurotoxin from cobras (*Naja* genus).

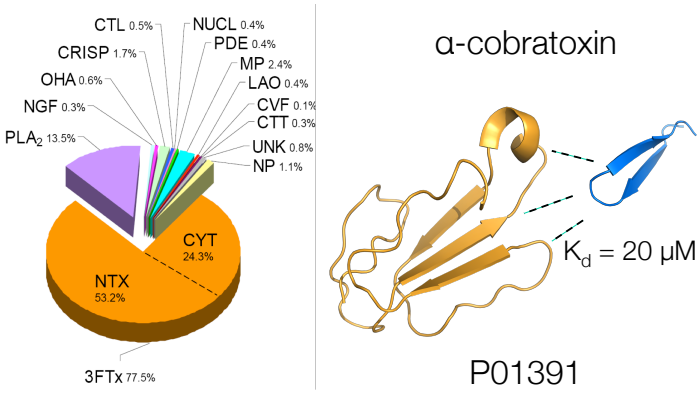

Figure 2: The high lethality of *Naja kaouthia* (Monocled cobra) venom is due to the high amount of α-neurotoxins, with the most abundant and toxic component being α-cobratoxin [2]. K_d was determined by Isothermal Calorimetry (ITC). Illustration of binding (binding place unknown).

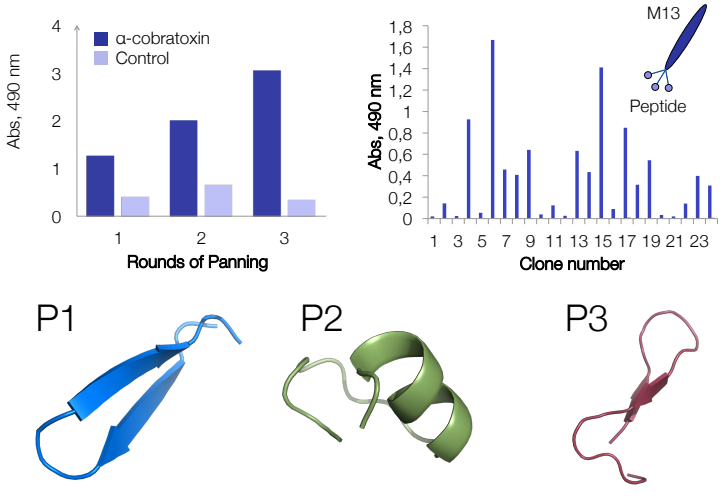

Figure 3: ELISA tests of panning rounds and selected monoclonal phage colonies. Phage display screening coupled to both normal sequencing of hits and next generation sequencing of panning rounds lead to the discovery of 3 peptides that interact with α-cobratoxin.

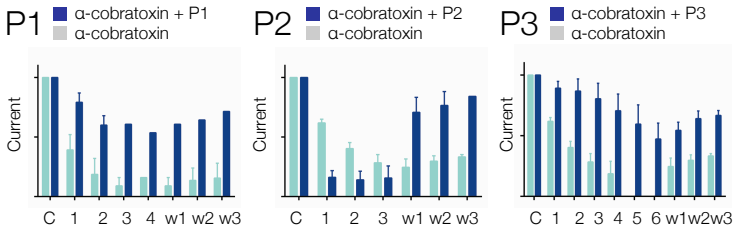

Figure 4: Peptides prevent α-cobratoxin from inhibiting nicotinic acetylcholine receptors in *Xenopus laevis* oocytes in two electrode voltage clamp (TEVC) experiments. 100 μM acetylcholine-gated currents were recorded alone (control, "C"); in the continued presence of either 40 nM α-cobratoxin alone (light blue bars, "1-3") or 40 nM α-cobratoxin and 100 μM peptide (dark blue bars, "1-3"); and then alone again (wash, "w1-w3"). P1 and P3 prevent the inhibition caused by α-cobratoxin, whereas P2 enhances both the onset and wash-out of inhibition.

Cross-reactive peptides for pan-specific antivenom

Given that other elapid venoms are rich in α-neurotoxins [3,4], the identified inhibitor may potentially provide protection against the neurotoxic effects exerted by α-neurotoxins present in a broad range of venoms.

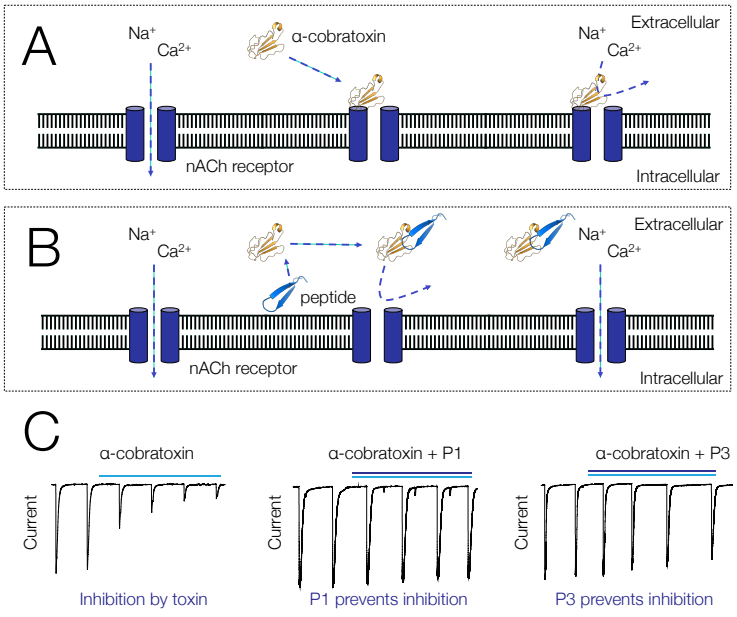

Figure 5: Schematic overview of physiological mechanism. A: α-cobratoxin inhibits the nicotinic acetylcholine receptor (nAChR) at the endplate of muscle fibers leading to flaccid paralysis. B: Peptides P1 and P3 bind to α-cobratoxin and prevent the toxin from inhibiting the nAChR. C: Measured ion currents through the nAChR in *Xenopus laevis* oocyte two electrode voltage clamp (TEVC) assay showing that peptides P1 and P3 prevent inhibition of ion current flow.

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